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Comparative Toxicity of Halogenated Hydrocarbons: Molecular Aspects

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THE COMPARATIVE HEPATOTOXICITIES OF PERFLUOROOCTANOIC ACID (PFOA), PERFLUORO-DECANOIC ACID (PFDA) AND THE TRI-AND TETRA-OLIGOMERS OF CHLORO-TRIFLUORO ETHYLENE (CTFE) HAVE BEEN INVESTIGATED IN THE RAT AND GUINEA PIG. ALL COMPOUNDS HAVE BEEN IDENTIFIED AS CAUSING HEPATOMEGALY, PEROXISOME PROLIFERATION AND CYTOCHROME P4504A1 INDUCTION IN THE RAT. THE GUINEA PIG IS NON-RESPONSIVE TO THESE COMPOUNDS AND THE OBSERVED LIVER CHANGES APPEAR TO BE SPECIFIC TO LOWER RODENT SPECIES. THE IMPLICATIONS OF THIS STUDY ARE THAT THE ABOVE COMPOUNDS DO NOT REPRESENT A HEALTH HAZARD TO MAN.

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1. OBJECTIVES

The comparative hepatotoxicity and enzyme induction potential of 4 peroxisome proliferators, namely perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA) and the tri-and tetra-oligomers of chlorotrifluooethylene (CTFE) were to be examined in experimental animals. These compounds are in use (or potential use) in the aerospace industry as fire retardants, surfactants, lubricants and corrosion inhibitors. Specifically, it was proposed to investigate the possibility that enzyme induction and hepatotoxicity are lower rodent-specific phenomena (the rat) and not seen in higher species (the guinea pig). In this way, the species differences in liver responses to these chemicals can be used to form part of a hazard identification and risk assessment to man.

2. STATUS OF THE RESEARCH EFFORT

The results of my studies are best described on a compound by compound basis as follows:

(i) PFOA

The influence of a single dose of the peroxisome proliferator PFOA on hepatic and renal mixed function oxidase activates were examined in the rat. Male Wistar albino rats (200-220g initial body weight) received a single i.p. injection of PFOA (75mg/kg). This dose level was chosen to minimise the "wasting syndrome" associated with this compound and pair-fed animals received only the dosing vehicle (propylene glycol: H_20, 50:50). Animals were individually housed in metabolism cages and body weight and food/water consumption were recorded daily. All animals were killed 3 days post-exposure and livers and kidneys removed, and tissue homogenates prepared for biochemical analyses.

Hepatomegaly, but not increased kidney weight, was observed in the PFOA group. In addition, the phenomenon of hepatic peroxisome proliferation was observed as assessed by increases in the marker enzymes palmitoyl CoA

oxidase (8-fold) and carnitine acetyl transferase (25-fold). The liver was also more susceptible than the kidney to PFOA-dependent induction of the 12-hydroxylation of lauric acid (2-fold), strongly suggesting induction of the CYP4A sub-family. This conclusion was further substantiated by Western blot analysis, wherein an anti-CYP4A1 antibody revealed a substantial PFOA-dependent induction of CYP4A1 in a pattern similar to that observed for the classical peroxisome proliferator, clofibrate. In addition, using a cDNA probe to CYP4A1 in Northern blot analysis, PFOA treatment resulted in a marked increase in the steady state level of CYP4A1 mRNA, again more extensively in liver than in kidney.

Taken collectively, this information confirms that PFOA is acting like a classical peroxisome proliferator, with the liver being the primary target tissue and the kidney being much less responsive.

(ii) TRI-CTFE AND TETRA-CTFE

Male Wistar rats were administered the CTFE oligomers, animals receiving 7 equimolar daily doses of the oligomers by oral gavage at a dose level of 2.3 m 'mol/kg. Animal monitoring and husbandry was as described above and liver/kidney homogenates prepared for biochemical analysis.

Both compounds caused significant hepatomegaly and induced the peroxisomal ß-oxidation of fatty acids, thus confirming these oligomers as peroxisome proliferators. Consistent with these conclusions, both the trimer and the tetramer increased the hydroxylation of lauric acid, indicating that the CTFEs were inducers of the CYP4A sub-family, a conclusion further supported by substantial increases in the steady state levels of the cognate CYP4A1 mRNA as determined by Northern blotting.

My data also indicates that the CTFE tetramer is a more potent enzyme inducer than the trimer and is consistent with their known hepatotoxicities. However, it must be emphasised that a more definative analysis of their relative potencies must await more extensive dose-response studies, and is the subject of my on-going work in hepatocyte monolayer primary culture studies.

For the CTFE oligomers, disposition and pharmacokinetic considerations make an important contribution to both their relative potencies as enzyme inducers and relative hepatotoxicities in that the tetramer is selectively retained in the liver to approximately double the liver concentrations achieved by the trimer.

(iii) PFDA

Male Wistar rats and male Duncan Hartley guinea pigs were dosed with one i.p. dose (20mg/kg) of PFDA, resulting in pronounced hepatomegaly in the rat but not the guinea pig. PFDA treatment also resulted in a 4-fold induction of lauric acid 12-hydroxylase activity in the rat but not the guinea pig, indicating induction of the CYP4A sub-family, a conclusion further substantiated by Western blot and Northern blot analyses.

Thus there is a clear species specific response to PFDA, with the rat and guinea pig being responsive and non-responsive species respectively.

3. RESEARCH PUBLICATIONS ARISING FROM THE GRANT

The following research papers have been submitted for publication (copies enclosed) and their current status indicated. These are

(i) Chlorotrifluoroethylene trimer and tetramer are inducers of the CYP4A sub-family. M. Diaz, E. Chinje, P. Kentish, B. Jarnot, M. George and G.G. Gibson (1993), Biochemical Pharmacology, in press.

(ii) Induction of the CYP4A sub-family by perfluorodecancio acid: the rat and the guinea pig as susceptible and non-susceptible species.

E. Chinje, P. Kentish, B. Jarnot, M. George and G.G. Gibson (1993), Toxicology Letters, in press.

(iii) Induction of cytochrome P4504A by the peroxisome proliferator perfluoro-n-octanoic acid. M. Diaz, E. Chinze, P.Kentish, B. Jarnot. M. George and G.G.Gibson (1993). Submitted to Toxicology, in press.

It should be noted that two USAF staff (B.Jarnot and M. George Dayton) were co-authors on the above three papers

In addition, some of the data described above have been incorporated into the following two publications:

(iv) Induction of cytochromes P450 by peroxisome proliferators.

G.G.Gibson, M. Diaz, E. Chinje and G.G.Gibson (1993), in Perosixome Proliferators : Unique Inducers of Drug-Metabolising Enzymes (D.Moody, Editor, CRC Press, in press.

(v) Review : Perosixome Proliferators, A Unique Set of Drug Metabolising Enzymes : Commentary on a Symposium

D.E. Moody, G.G.Gibson, D.F. Grant, J.Magdalou and M.S. Rao (1992). Drug Metabolism and Disposition, **20**, 779-791.

4. PERSONNEL ASSOCIATED WITH THE RESEARCH EFFORT

(i) Prof.G.Gordon Gibson, Principal Investigator and Laboratory Task Manager.

(ii) Dr Edwin Chinje, Postdoctoral Fellow hired on current grant.

(iii) Dr Maria Diaz, Visiting Scientist from the University of Pamplona, Spain

(iv) Mr Peter Kentish, Research Technician, University of Surrey

(v) Dr Bruce Jarnot, USAF Wright-Patterson AFB, Dayton, Ohio.

(vi) Ms Marilyn George, USAF Wright-Patterson AFB, Dayton, Ohio

5. INTERACTIONS

(i) Response of Cytochrome P450s to Peroxisome Proliferators. Invited Platform presentation, ASPET Symposium in FASEB Spring meeting, Anaheim,California, USA, April 1992.

(ii) Peroxisome Proliferation and Non-mutagenic Hepatocarcinogenesis. Lecture given in Conference on Applications of Advances in Toxicology to Risk Assessment, Sponsored by the US Air Force, Wright-Patterson AFB, Dayton, Ohio, USA, May 1992.

(iii) Biochemistry and Molecular Biology of Peroxisome Proliferation. Invited lecture at Annual Meeting of the Biological Society of Chile, Termas de Paychue, Chile, November, 1992

(iv) Peroxisome proliferation : Toxicological Implications. Departmental Seminar, University of Birmingham, England, November 1992

(v) Non-mutagenic carcinogens. Invited lecture at the UK Environmental Mutagenicity Meeting, Guildford, March 1993.

6. INVENTIONS AND PATENTS.

Not applicable